METHODS OF OBTAINING A MIXED POPULATION OF HUMAN XCR1+ AND PLASMACYTOID DENDRITIC CELLS FROM HEMATOPOIETIC STEM CELLS

FIELD OF THE INVENTION

[0001] The present invention relates to methods of obtaining a mixed population of human XCR1$^+$ and plasmacytoid dendritic cells from hematopoietic stem cells.

BACKGROUND OF THE INVENTION

[0002] Dendritic cells (DC) are a heterogeneous family of rare leukocytes that sense danger signals and convey them to lymphocytes for the orchestration of adaptive immune defenses.

[0003] Clinical trials used monocytes derived DC (MoDC) to attempt to promote protective immunity in patients suffering from infections or cancer. These immunotherapies showed limited efficacy, owing to the poor recirculation of MoDC to lymph nodes (Adema, G J, et al. Migration of dendritic cell based cancer vaccines: in vivo veritas? Curr Opin Immunol. 2005; 17:170-174) (Plantinga, M et. al.. Conventional and Monocyte-Derived CD11b(+) Dendritic Cells Initiate and Maintain T Helper 2 Cell-Mediated Immunity to House Dust Mite Allergen. Immunity. 2013) and likely to other yet uncharacterized functional differences between MoDC and lymphoid tissues-resident DC (LT-DC). Hence, major efforts are being made to better characterize human LT-DC and to evaluate their immunoactivation potential. Steady state human blood and secondary lymphoid organs contain three major DC subsets, CD141(BDCA3)$^+$CLEC9A$^+$ classical DC (cDC), CD1c (BDCA1)$^+$ cDC and CLEC4C(BDCA2)$^+$ plasmacytoid DC (pDC) (Ziegler-Heitbrock, L et al. Nomenclature of monocytes and dendritic cells in blood. Blood. 2010; 116:e74-80). Homologies exist between mouse and human LT-DC subsets (Robbins, S H, et al. Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. Genome biology. 2008) (Crozat, K, et al. Comparative genomics as a tool to reveal functional equivalences between human and mouse dendritic cell subsets. Immunological reviews. 2010). Comparative transcriptomics (Watchmaker, P B, et al. Comparative transcriptional and functional profiling defines conserved programs of intestinal DC differentiation in humans and mice. Nat Immunol. 2014) (Haniffa, M, et al. Human tissues contain CD141hi cross-presenting dendritic cells with functional homology to mouse CD103+ nonlymphoid dendritic cells. Immunity. 2012; 37:60-73) and functional studies (Crozat, K, et al. The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8alpha+ dendritic cells. J Exp Med. 2010; 207:1283-1292.) (Bachem, A, et al. Superior antigen cross-presentation and XCR1 expression define human CD11c+ CD141+ cells as homologues of mouse CD8+ dendritic cells. J Exp Med. 2010; 207:1273-1281) (Jongbloed, S L et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. J Exp Med. 2010; 207:1247-1260) showed that human CD141$^+$CLEC9A$^+$ cDC are homologous to mouse spleen CD8α^+ cDC, which are specialized in cross-presentation. Mouse CD8α^+ cDC and human CD141$^+$ CLEC9A$^+$ cDC specifically express the XCR1 chemokine receptor (Dorner, B G et al. Selective expression of the chemokine receptor XCR1 on cross-presenting dendritic cells determines cooperation with CD8+ T cells. Immunity. 2009; 31:823-833) (Crozat, K, et al. Cutting edge: expression of XCR1 defines mouse lymphoid-tissue resident and migratory dendritic cells of the CD8alpha+ type. J Immunol. 2011; 187:4411-4415) and can therefore be coined XCR1$^+$ cDC. The ligands of XCR1 are selectively expressed in Natural Killer (NK) and CD8 T cells, promoting their interactions with XCR1$^+$ cDC. Human XCR1$^+$ cDC have been described in many tissues (Yoshio, S et al. Human blood dendritic cell antigen 3 (BDCA3)(+) dendritic cells are a potent producer of interferon-lambda in response to hepatitis C virus. Hepatology. 2013; 57:1705-1715). Human and mouse XCR1$^+$ cDC specifically express high levels of Toll-like receptor (TLR)-3 (Crozat, K, Vivier, E, Dalod, M. Crosstalk between components of the innate immune system: promoting anti-microbial defenses and avoiding immunopathologies. Immunological reviews. 2009; 227:129-149) and respond to its triggering with hepatitis C virus or with the synthetic ligand polyinosinic-polycytidylic Acid (PolyL-C) by interferon (IFN)-λ production (Zhang, S et al. Human type 2 myeloid dendritic cells produce interferon-lambda and amplify interferon-alpha in response to hepatitis C virus infection. Gastroenterology. 2013; 144:414-425 e417) and by enhanced cross-presentation. The extent to which human XCR1$^+$ cDC are more efficient for cross-presentation than other human DC subsets is debated. It depends on the tissue origin of the DC subsets, on their activation status and on the mode of antigen delivery (Segura, E et al. Similar antigen cross-presentation capacity and phagocytic functions in all freshly isolated human lymphoid organ-resident dendritic cells. J Exp Med. 2013; 210:1035-1047) (Cohn, L, et al. Antigen delivery to early endosomes eliminates the superiority of human blood BDCA3+ dendritic cells at cross presentation. J Exp Med. 2013; 210:1049-1063) (Flinsenberg, T W, et al. Fcgamma receptor antigen targeting potentiates cross-presentation by human blood and lymphoid tissue BDCA-3+ dendritic cells. Blood. 2012; 120:5163-5172). However, several independent studies showed that human XCR1$^+$ blood cDC (bcDC) excel at cross-presentation of cell-associated antigens and of particulate antigens delivered through Fcγ receptors, through lysosomes or upon PolyL-C stimulation (Nizzoli, G et al. Human CD1c+ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T cell responses. Blood. 2013). Since they share unique characteristics with mouse XCR1$^+$ cDC, human XCR1$^+$ bcDC constitute a distinct human DC subset that may have potential clinical applications (Gallois, A, Bhardwaj, N. A needle in the 'cancer vaccine' haystack. Nat Med. 2010; 16:854-856) (Radford, K J, Caminschi, I. New generation of dendritic cell vaccines. Hum Vaccin Immunother. 2013; 9) (Tacken, P J, Figdor, C G. Targeted antigen delivery and activation of dendritic cells in vivo: steps towards cost effective vaccines. Semin Immunol. 2011; 23:12-20). Accordingly there is a need for having in vitro method of obtaining such cells. Recently, the inventors described a protocol for the in vitro generation of human XCR1$^+$ cDC from CD34$^+$ hematopoietic progenitors (Balan S, Dalod M. In Vitro Generation of Human XCR1(+) Dendritic Cells from CD34(+) Hematopoietic Progenitors. Methods Mol Biol. 2016; 1423:19-37. doi: 10.1007/978-1-4939-3606-9_2). Immunotherapy with autologous human pDC directly isolated ex vivo, loaded in vitro with antigens and matured